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TITLE: P53 Mutation Analysis to Predict Tumor Response in Patients Undergoing Neoadjuvant Treatment for Locally Advanced Breast Cancer

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The two most effective classes of chemotherapeutic drugs in breast cancer are the anthracyclines and the taxanes, which differ in mechanisms of action and resistance. P53 may mediate responsiveness to these drugs. P53 mutations status has had limited usefulness as a predictive tumor marker given the technical complexity of previous methods to determine it, however the development of p53 GeneChip technology has made high-throughput mutation analysis more feasible.

In an ongoing multiinstitutional prospective trial that is not supported by this award, breast cancer patients receiving neoadjuvant chemotherapy using these drugs have serial response assessments and tumor sampling for research purposes. This project involves analyzing he banked tumor specimens for p53 mutations using the GeneChip method. We hypothesize that p53 status of the primary tumor will predict response to anthracycline-based and taxane-based chemotherapy given at different times in the same patient. Progress to date includes optimizing the GeneChip method of p53 mutation analysis for core biopsy specimens, successful scaling down of DNA requirements for such assays allowing evaluation of small tumor biopsy samples, and optimizing methods for p53 amplification within 1-2 large fragments so that SSCP and sequencing analysis will be feasible despite the small amount of DNA available.

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Introduction

The two most effective classes of chemotherapeutic drugs in breast cancer are the anthracyclines and the taxanes, which differ in mechanisms of action and resistance. Responsiveness to anthracyclines and taxanes may be mediated in part by the p53 mutational status of the tumor. P53 mutation status has had limited usefulness as a predictive tumor marker given the technical complexity of previous methods to determine it, however the development of p53 GeneChip technology has made high-throughput mutation analysis more feasible. This technology has been successfully applied to human tumor specimens (1,2). Dr. Conway Dorsey's laboratory has previously determined the spectrum of expected p53 mutations in breast cancer (3) using sequencing, and is performing the GeneChip analysis and sequencing in this study.

An ongoing multiinstitutional prospective trial, breast cancer patients who are receiving neoadjuvant chemotherapy have serial response assessments performed and undergo sampling of their tumor for research purposes at three time points. These timepoints are: 1) prior to any chemotherapy, 2) following treatment with an anthracycline-containing regimen. Those that receive a subsequent chemotherapy have another sample obtained after that regimen. This project involves analyzing the banked specimens for p53 mutation status using the GeneChip method. We hypothesize that p53 status of the primary tumor will predict response to anthracycline-based and taxane-based chemotherapy given at different times in the same patient.

Body

This award is for performance of laboratory assays upon banked tumor specimens obtained from ongoing correlative science trials that are funded through alternative mechanisms. The performance of those trials, however, is crucial to the outcome of this project, so is summarized here. The trials, Lineberger Comprehensive Cancer Center (LCCC) Project 9819 and Cancer and Leukemia Group B (CALGB) Protocol 150007, are both open and enrolling patients. LCCC 9819 at this time has enrolled 66 patients, and CALGB 150007 46 patients. Tumor tissues from these protocol patients have been banked, and have not yet been tested. Both of these prospective trials are supported by the National Cancer Institute Specialized Programs of Research Excellence awards given to several collaborating institutions.

Statement of Work

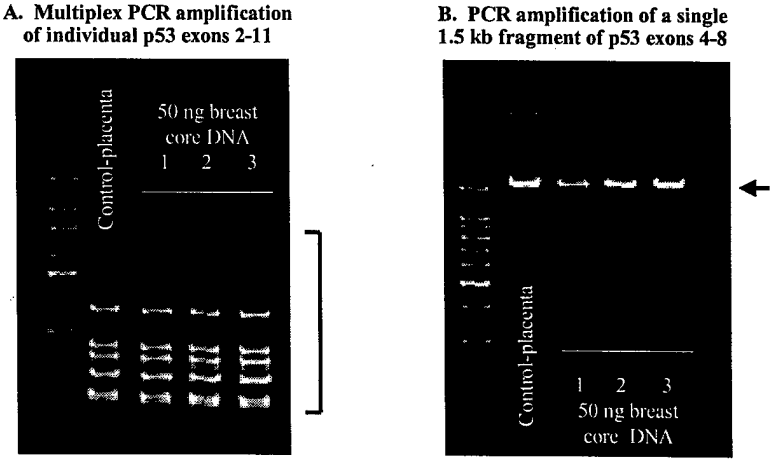
Progress upon the approved statement of work for year 1 is outlined below in the format used in the original application.

Task 1. To optimize the GeneChip method of p53 mutation analysis in the UNC Molecular Epidemiology Core Laboratory (months 1-6)

This portion of the research involved the establishment of multiplex PCR conditions to co-amplify all p53 exons from within one reaction, and the optimization of the p53 GeneChip hybridization conditions and analysis of microarray data. The p53 GeneChip assay has been optimized using the Affymetrix normal control DNA (human placental DNA) and cell lines (BT549, Bt474, MDA-MB-231, and MDA-MB-435), and has been successfully applied to human breast cancer core biopsy specimens.

Because of the very small quantities of DNA expected from the breast core biopsies, our first priority in establishing assay conditions for the p53 GeneChip assay was to determine the smallest amount of DNA that could be reasonably amplified from the cores, but that would provide a valid p53 mutation result. This is crucial because only 8-10mg of total tissue is obtained from each core biopsy, with nucleic acid-based studies planned by several collaborating laboratories. Using the above cell lines, the DNA concentration required for the multiplex p53 PCR reaction, which amplifies each p53 exon 2-11 in individual fragments, has been successfully reduced from an original amount of 250ng DNA down to 50ng DNA. This reduction of DNA has not compromised our ability to identify mutations within cell line DNAs. Five test frozen breast core DNA samples (#1-03-0077 UNC, #2- B1117 T4BRLT, #3- 43957899) have been received from a collaborating institution, the University of California at San Francisco (UCSF) and analyzed using the P53 Gene Chip assay and only 50ng of DNA (Figure 1, see attached). Multiplex PCR amplification of p53 exons 2-11 from three test cores is shown in Figure 2A. Breast core DNA sample #2 (B1117 T4BRLT) showed a

Figure 2: PCR Amplification of DNAs Extracted from Frozen Test Core Breast Biopsies*



Core 1 = UNC 03-0077
Core 2 = B1117T4BRLT
Core 3 = 43957877

*Using the CGH DNA Isolation Protocol

mutation in exon 7 at codon 234 (TAC>TAA). The test core DNAs were also screened in a large fragment PCR reaction of the P53 gene, amplifying exons 4-8 in a single fragment. This reaction was successful indicating that it would be possible to amplify large fragments of DNA prior to performing P53 mutation screening if necessary (Figure 2B). The research plan includes single strand conformational polymorphism (SSCP) and sequencing analysis to comprehensively identify p53 mutations in GeneChip-negative samples. This technique of large-fragment PCR upon these limited tissue samples will allow the SSCP and mutation analysis to be performed with a minimal of required DNA.

Task 2. To determine the p53 mutational status of the primary breast cancers before any chemotherapy. In cases whose tumors exhibited p53 mutations pre-chemotherapy, determine if the same mutations are detectable after anthracycline then again after taxane with or without trastuzumab (Months 6-36).

Dr. Perou's laboratory was originally planned to obtain large molecular weight genomic DNA from frozen breast tumor pretherapy core biopsies (timepoint 1) from the sample processed for gene expression array analysis. This aim has been modified to include two investigators from other institutions who are also using nucleic acids from the same banked tissues, Dr. Joe Gray and Dr. Chris Haqq of UCSF. Those studies are supported under a different mechanism. Dr. Gray and Dr. Haqq are performing complementary assays upon the same tissue. Dr. Gray's laboratory is performing comparative genomic hybridization (CGH) studies looking for genome-wide deletions and amplifications. Dr. Haqq's laboratory is working with Dr. Perou's to optimize the gene expression analysis. Recent studies have demonstrated that global DNA copy number has a strong influence upon the expression patterns seen in breast cancer (4),

making this complementary analysis very timely. For this reason, a great deal of effort has been made to minimize the tissue, DNA, and RNA needs of each group so that all the planned assays may be performed. In order to optimize the conditions for maximal nucleic acid retrieval from these limited tissue resources, a training set of biopsies is being obtained and tested. Using a method with alternate sections being used for RNA and DNA, the following results have been obtained, suggesting that there will be adequate DNA for the planned p53 assay as well as the other nucleic acid-based studies (Table 1).

Table 1. Nucleic acids yield from training set test cores using updated tissue processing protocol*.

Source	Name	Total RNA (μg)	Total DNA (μg)
UNC	UNC 03-0077 14G	15.64	6.18
UNC	UNC 03-0306B 14G	0.375	2.09
MSK	MSK 14G	11.88	2.99
MSK	MSK 16G	6.76	2.96
UCSF	UCSF B1131 14G	1.45	2.60
UCSF	UCSF B1131 16G	1.35	2.60
UW	UW subject 1 core 2	3.77	4.07
UW	UW subject 2 core 2	7.66	5.16
UW	UW subject 3 core 2	1.53	1.77

* to August, 2003

We have also examined the proportion of invasive tumor cells within the biopsy in hematoxylin and eosin-stained sections from the formalin-fixed, paraffin-embedded biopsies that were obtained at the same time as the frozen samples that will be tested in this project. This preliminary analysis from 41 of the research samples from UNC-LCCC Project 9819 reveals that of 41 core biopsies, 28 (68%) had at least 20% tumor cells throughout the core. Thus, in most cases, the unenriched pretreatment core will have sufficient tumor cells for analysis. For the cancers with lower ratios of tumor cells: normal, we are considering various ways in which the proportion of tumor can be

enriched or that normal cells can be removed from the tissue sample before nucleic acid retrieval. Since p53 mutations represent qualitative differences, our project may be less impacted than the more quantitative assays such as CGH and expression arrays by these issues.

Task 3. To correlate p53 status with response to anthracycline chemotherapy, then taxane with or without trastuzumab in the same patient (months 30-36):

This aim begins in year 3.

Task 4. To compare p53 status with results of other planned assays within the larger correlative science trial such as bcl-2, estrogen receptor, and gene expression array analysis (months 1-36).

In the upcoming year, the training set will be completed, and we will move to examining the banked specimens for p53 point mutations and single base deletions using the Affymetrix GeneChip. This will allow performance of the planned correlations with other biomarkers. Notably, in its role as the supporting institution for the prospective trials, the National Cancer Institute has initiated the development of a database that will allow multiple investigators involved in this study to cross-examine the relationships of various markers to each other and to outcome.

Task 5. To functionally classify the p53 mutants identified in breast cancer using established and newly developed yeast-based assays (Months 12-36).

This aim does not begin until year 2.

Key Research Accomplishments

- Establishment of conditions for Affymetrix GeneChip assay using frozen breast cancer tissue as starting material as well as cell line DNA. Test samples have identified p53 mutations in frozen tumors.
- Scaling down DNA requirements. The recommended amount of DNA for the GeneChip is 250 ng. In Dr. Dorsey's laboratory, conditions have been optimized for successful assay using only 50ng
- Demonstration of successful p53 amplification within 1-2 large fragments (1.5 kb) for PCR. This will allow performance of single strand conformational polymorphism (SSCP) and sequencing analysis in GeneChip-negative samples in spite of small amount of DNA available from these limited tissue resources.

Reportable Outcomes

The methodologic issues surrounding the optimal use and processing of core biopsy specimens for correlative science will likely be reported early in the course of this award. At this time, while they have been discussed in closed meetings, they have not been submitted for presentation or publication due to the preliminary nature of the current analysis.

Conclusions

Ascertainment of tumor samples from patients undergoing neoadjuvant chemotherapy for locally advanced breast cancer is continuing. In addition to the UNC samples, the NCI-supported multiinstitutional study began in the fall 2002, and has already accrued 46 patients. This confirms that the investigators will have the tissue resources to perform the planned analysis of p53 as a predictive marker in breast cancer. At the current rate of accrual, the prospective trials will complete on time. Preliminary data from test core biopsies from non-protocol patients suggests that the planned assays that will complement the p53 analysis are feasible despite the small amount of tissue available.

The GeneChip method of p53 mutation analysis has been optimized in Dr. Conway Dorsey's laboratory. Moreover, her laboratory has successfully reduced the required amount of DNA to 50 ng. This preliminary work suggests that her laboratory will be able to successfully perform the GeneChip analysis upon the amount of nucleic acids available from the core biopsies obtained in the clinical trials. Finally, in order to maximize the sensitivity of screening for p53 mutations, the research plan included SSCP and sequencing for GeneChip-negative tumors. Dr. Conway Dorsey's laboratory has developed a technique for large-fragment PCR that will permit this additional evaluation in the limited DNA available in these samples.

References

1. Ahrendt SA, Hu Y, Buta M et al.: p53 mutations and survival in stage I non small-cell lung cancer: results of a prospective study. J Natl Cancer Inst 2003;95(3):961-970

2. Takahashi Y, Ishii Y, Nagata T et al.: Clinical application of oligonucleotide probe array for full-length gene sequencing of TP53 in colon cancer. Oncology 2003;64(1):54-60

3. Conway K, Edmiston S, Cui L et al.: Prevalence and spectrum of p53 mutations associated with smoking in breast cancer. Cancer Res 2002;62(7):1987-1995

4. Pollack JR, Sorlie T, Perou CM et al.: Microarray analysis reveals a major direct role of DNA copy number alteration in the transcriptional program of human breast tumors. Proc Natl Acad Sci USA 2002;99(20):12963-12968

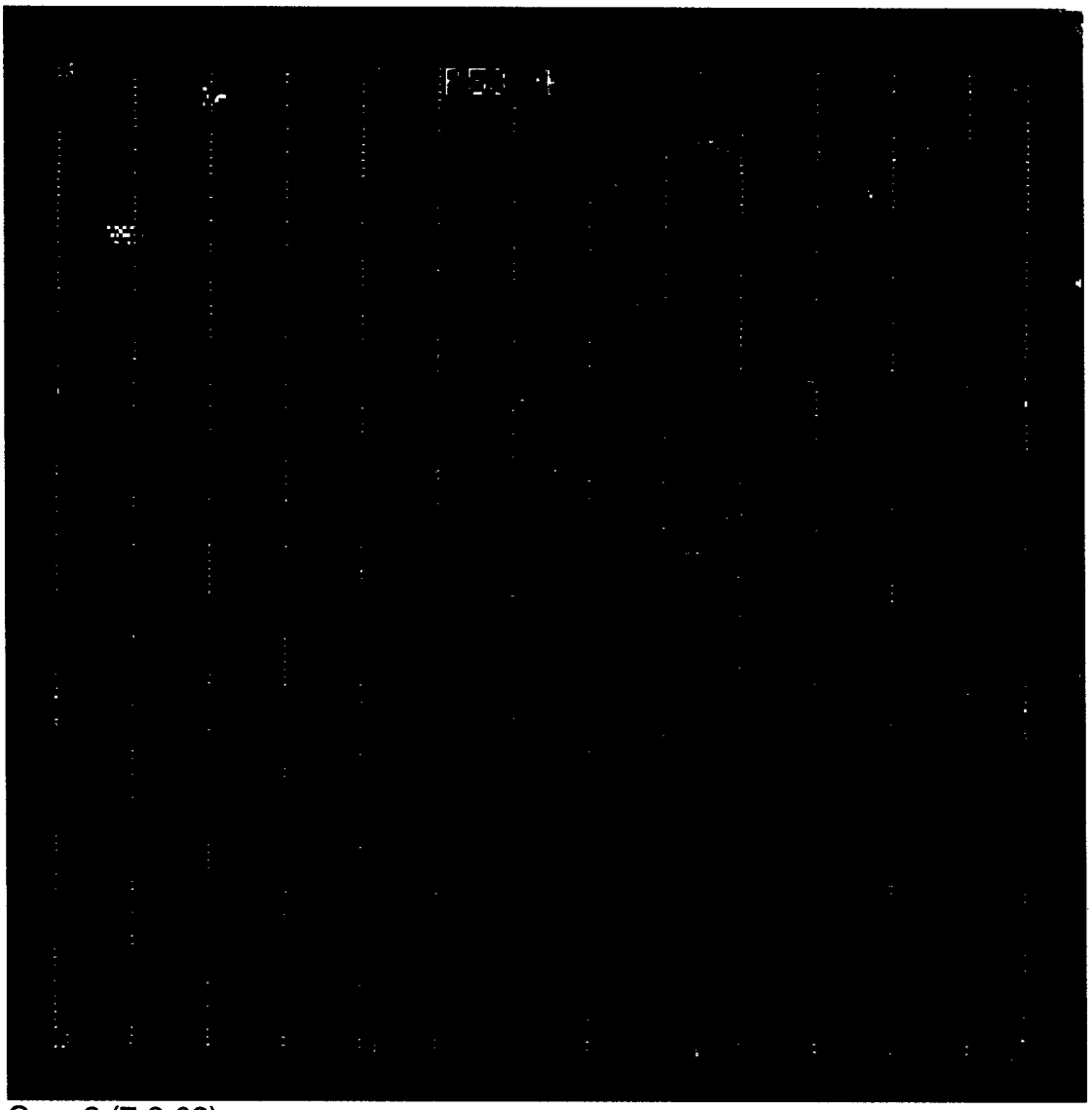


Figure 1. p53 geneChip using 50 ng of DNA from a core biopsy of a human breast cancer.